REMARKS

Claims 1-3, 6-8 and 23-28 are pending in the Subject Application. In the Office Action of July 31, 2009, claims 1-3, 6-8, and 23-28 stand rejected. Claim 1 has been amended to recite, "[a] method of expanding a population of human stem cells by promoting self-renewal of the population of human stem cells comprising: delivering small RNA interfering sequences (siRNA) to the human stem cells for the reduction of p18 levels in the intracellular environment of the stem cells" and finds support throughout the specification, such as, page 1, lines 15-18; page 5, lines 21-22; page 23, lines 22-23; and page 24, lines 22-25. Claim 23 has been amended to recite, "[a] method of stimulating self-renewal of a population of human stem cells by reducing intracellular levels of p18 comprising: delivering small RNA interfering sequences (siRNA) to the human stem cells by one of electroporation or lentiviral vector for the reduction of p18 levels in the intracellular environment of the stem cells" and finds support throughout the specification, such as, page 1, lines 15-18; page 5, lines 21-22; page 23, lines 22-23; and page 24, lines 22-25. Claims 2 and 24 have been amended to recite, "wherein said human stem cells are adult stem cells" and find support throughout the specification, such as, page 1, lines 19-20; page 5, lines 21-22; and page 23, lines 22-23. Claims 6 and 26 have been amended to recite, "further comprising implanting the siRNA treated human stem cells into a human" and find support throughout the specification such as page 5, lines 21-22 and page 24, lines 22-25. Claims 7 and 27 have been amended to recite, "wherein said siRNA treated human stem cells are adult stem cells" and find support throughout the specification, such as, page 5, lines 21-22; page 23, lines 22-23, and page 24, lines 22-25. Claims 3, 8, 25,

and 28 have been canceled. Claims 4-5 and 9-22 were previously canceled. New claims 29-39 have been added and find support throughout the specification, such as, page 1, lines 15-18; page 2, lines 10-11; page 4, lines 1-2; and page 24, line 15 to page 25, line 13.

In addition, the specification has been amended to include several paragraphs to further clarify the multiple figures in Figures 1-4. The amendment to the specification finds support in the original Figures 1-4, original description of Figures 1-4 on page 4, lines 5-15, and in Examples 1-8.

Applicants respectfully submit that no new matter has been introduced by the amendments to the claims, addition of new claims, and amendment to the specification.

A) Objection to Specification

The specification has been objected to for failing to contain a reference to each of the drawing figures as required by 37 C.F.R. 1.74. Specifically, the "Brief Description of the Figures" at pages 4-6 is incomplete with respect to the description of each individual figure in Figures 1-4.

Applicants have amended the specification to include descriptions for Figures 1a, 1b, 2a, 2b, 2c, 3a, 3b, 3c, 3d, 4a, and 4b. Accordingly, withdrawal of the objection to the specification is respectfully requested.

Hence, Applicants respectfully submit that the objection is obviated and request that this objection be withdrawn.

B) Rejection of Claims 1-3 and 6-8 under 35 U.S.C. §112, first paragraph

Claims 1-3 and 6-8 stand rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the enablement requirement. Specifically, the Examiner asserts that the specification is found to be not enabling for "controlling" self-renewal by reducing intracellular levels of p18 through the use of siRNA (Office Action, page 6). Applicants respectfully disagree. However, in order to expedite prosecution of this matter, claim 1 has been amended to replace "controlling" with "expanding a population of human stem cells by promoting". In addition, claims 3 and 8 have been canceled.

Thus, Applicants submit that sufficient enablement has been provided by the amendment to claim 1. Accordingly, withdrawal of the rejection of claims 1-3 and 6-8 under 35 U.S.C. §112, first paragraph is respectfully requested.

Hence, the Applicants respectfully submit that the rejection of claims 1-3 and 6-8 is obviated and requests that this rejection be withdrawn.

C) Rejection of Claims 1-3, 6-8 and 23-28 under 35 U.S.C. §112, first paragraph

Claims 1-3, 6-8 and 23-28 stand rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. Specifically, the Examiner asserts that the specification only provides evidence that reducing p18 using siRNA results in increased self-renewal in hematopoietic stem cells (HSCs) (Office Action, page 5, second paragraph). Applicants respectfully disagree.

Applicants submit that the specification states,

> [d]ownmodulating p18 may permit enhanced stem cell expansion in-vitro, a method that can be used in stem cell expansion and in defining other active agents for stem cell expansion. Given the non-specific expression

of p18 in hematopoietic cells, this approach can also be applied to other stem cells in the body (specification, page 3, line 23 to page 4, line 2)

The Examiner further asserts "a large amount of undue experimentation would be required to determine the parameters and additional conditions necessary to successfully promote self-renewal of all stem cells" (Office Action, page 10, second paragraph). Applicants also respectfully disagree. Applicants submit that p18 acts as an inhibitor at the early G1-phase of the cell cycle (See page 2 of specification, first paragraph). Applicants further submit that all types of adult human stem cells (hematopoietic and non-hematopoietic stem cells) go through the four distinct phases of a cell cycle. Therefore, p18 normally acts as an inhibitor at the early G1-phase for all types of adult stem cells. The fact that p18 plays a role in both hematopoietic and non-hematopoietic stem cells demonstrates that one of ordinary skill in the art would expect to be able to use the claimed method to promote or stimulate self-renewal in all human stem cells. Thus, no undue experimentation would be necessary for one of ordinary skill in the art. Thus, Applicants have amended claims 1, 2, 6-7, 23-24, and 26-27 to recite "human stem cells". In addition, claims 3, 8, 25 and 28 have been canceled.

Thus, Applicants submit that sufficient enablement has been provided by the amendment to claim 1, 2, 6-7, 23-24 and 26-27. Accordingly, withdrawal of the rejection of claims 1-3, 6-8, and 23-28 under 35 U.S.C. §112, first paragraph is respectfully requested.

D) Rejection of Claims 2, 7, 24, and 27 under 35 U.S.C. §112, second paragraph

Claims 2, 7, 24, and 27 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the Applicants regard as the invention. Specifically, the Examiner asserts that "predominantly undifferentiated stem cells" infer that not all stem cells need to be undifferentiated.

Applicants submit that claims 2, 7, 24 and 27 have been amended to replace "predominately undifferentiated stem cells" with "adult stem cells". Accordingly, withdrawal of the rejection of claims 2, 7, 24 and 27 under 35 U.S.C. §112, second paragraph, is respectfully requested.

E) Rejection of Claims 6 and 26 under 35 U.S.C. §112, second paragraph

Claims 6 and 26 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the Applicants regard as the invention. Specifically, the Examiner asserts that claims 6 and 26 should recite "implanting the human...". Applicants agree. Applicants submit that claims 6 and 26 have been amended to recite "implanting the human...". Accordingly, withdrawal of the rejection of claims 6 and 26 under 35 U.S.C. §112, second paragraph, is respectfully requested.

F) Rejection of Claims 6 and 26 under 35 U.S.C. §112, second paragraph

Claims 6 and 26 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which

the Applicants regard as the invention. Specifically, the Examiner asserts that claims 6 and 26 lack antecedent basis for the limitation "the implanted human stem cells". Applicants submit that claims 6 and 26 have been amended to delete "wherein the implanted human stem cells are self-renewing".

Accordingly, withdrawal of the rejection of claims 6 and 26 under 35 U.S.C. §112, second paragraph, is respectfully requested.

G) Rejection of Claims 1-3 and 23-25 under 35 U.S.C. §103(a)

Claims 1-3 and 23-25 stand rejected under 35 U.S.C. § 103(a) for assertedly being obvious in view of U.S. Patent No. 6,033,847 to Sherr *et al.* (hereinafter "Sherr") further in view of Bertrand, J.R., "Comparison of antisense oligonucleotide and siRNAs in cell culture and in vivo", Biochemical and Biophysical Research Communications, 2002, 296: pages 1000-1004 (hereinafter, "Bertrand") further in view of An, D.S., "Efficient lentiviral vectors for short hairpin RNA delivery into human cells", Human Gene Therapy, 2003, 14: pages 1207-1212 (hereinafter, "An") further in view of Walters, D.K., "The effectiveness of double-stranded short inhibitory RNAs (siRNAs) may depend on the method of transfection", Antisense and Nucleic Acid Drug Development, 2002, 12: pages 411-418 (hereinafter, "Walters"). Applicants traverse this rejection for at least the reasons set forth herein.

To determine the obviousness of a claim, an Examiner must make "a searching comparison of the claimed invention – *including all its limitations* – with the teaching of the prior art." *In re Ochiai*, 71 F.3d 1565, 1572 (Fed. Cir. 1995) (emphasis added). Thus, "obviousness requires a suggestion of all limitations in a claim." *CFMT, Inc. v.*

Yieldup Intern. Corp., 349 F.3d 1333, 1342 (Fed. Cir. 2003) (*citing In re Royka*, 490 F.2d 981, 985 (CCPA 1974)). Furthermore, as set forth in MPEP §2142, the key to supporting any rejection under 35 U.S.C. § 103(a) is the clear articulation of the reason why the claimed invention would have been obvious. As the Supreme Court recently stated, "*there must be some articulated reasoning* with some rational underpinning to support the legal conclusion of obviousness." *KSR Int'l v. Teleflex Inc.*, 127 S. Ct. 1727, 1741 (2007) (quoting In re Kahn, 441 F.3d 977, 988 (Fed. Cir. 2006)) (emphasis added).

For example, where the Examiner contends that the claimed method of promoting or stimulating self-renewal is obvious because of the elements gleaned from the combined teachings of Sherr, Bertrand, An, and Walters, the Examiner must explain why it would have been obvious to one of ordinary skill in the art to arrive at the claimed method. Applicants respectfully submit, however, that the Examiner has failed to clearly articulate the reasoning in support of the asserted conclusion of obviousness in view of the combined teachings of Sherr, Bertrand, An, and Walters and that there is no reason why one of ordinary skill in the art would arrive at the claimed method. This is so because there is nothing in Sherr, Bertrand, An, or Walters that would teach or suggest a method of promoting or stimulating self renewal as set forth below in claims 1 and 23.

Claim 1 of the Subject Application sets forth a method of expanding a population of human stem cells by promoting self-renewal of the population of human stem cells comprising: delivering small RNA interfering sequences (siRNA) to the human stem cells for the reduction of p18 levels in the intracellular environment of the stem cells.

Claim 23 of the Subject Application sets forth a method of stimulating self-renewal of a population of human stem cells by reducing intracellular levels of p18 comprising: delivering small RNA interfering sequences (siRNA) to the human stem cells by one of electroporation or lentiviral vector for the reduction of p18 levels in the intracellular environment of the stem cells.

The Examiner asserts that "proliferation is not necessarily differentiation" and "...proliferation, by definition in the field of cell biology, is increased cell number by division" (Office Action, page 15, second paragraph). Applicants respectfully disagree.

Applicants submit that cell proliferation may be defined as increasing cell number by division in cell biology. However, in stem cell biology, stem cell proliferation refers to cell division and differentiation of stem cells into progenitor cells. For example, stem cell proliferation results in one stem cell becoming two progenitor cells. In contrast, self renewal results in either one stem cell becoming two stem cells or one stem cell becoming one stem cell and one progenitor cell. Thus, self-renewal expands a population of stem cells. In contrast, stem cell proliferation will never expand a population of stem cells and will eventually eliminate the entire population of stem cells once the population becomes entirely progenitor cells.

Furthermore, the Examiner asserts that "antisense oligonucleotides and siRNA molecules were both recognized as effective agents to inhibit gene expression in mammalian cells by degrading targeted messenger RNA, while the mechanisms of action differ, the end result is equivalent" (Office Action, page 14). Applicants respectfully disagree.

Applicants submit that both siRNA and antisense oligonucleotides do indeed degrade targeted messenger RNA. However, siRNA and antisense oligonucleotides are very different. For example, they have totally distinct structures, processing pathways, mechanisms of action (See appendix A: Hall, Nature Reviews Genetics, 2004; Appendix B: Scherer and Rossi, Nature Biotechnology, 2003; Appendix C: Castanotto and Rossi, Nature, 2009) and most importantly very different levels of inhibition. Applicants further submit that the silencing of gene expression by siRNA is much more potent than antisense oligonucleotides. For example, Figures 2 and 3 of Bertrand, demonstrate that siRNA inhibits GFP expression more efficiently (80%) compared to antisense oligonucleotide inhibition of GFP (only 20%). Thus, Applicants submit that the end result (i.e., the inhibition of gene expression) is not equivalent. Bertrand further supports this notion by stating:

> [t]he siRNA appears to be more efficient than the antisense oligonucleotides at 5 h incubation since we obtain around 80% inhibition of GFP (page 1002, second column, lines 2-4).
>
> [a]t a 20 h incubation, the effect is more dramatic, since the inhibition triggered by siRNAs increases. We know that the antisense oligonucleotides has no effect after a 20 h incubation (page 1002, second column, lines 5-8).
>
> [i]t appears that the effect triggered by siRNA is higher and lasts longer (page 1002, second column, lines 10-12).

Thus, Applicants submit that Sherr's method of stimulating cell proliferation using antisense oligonucleotides will not lead to self-renewal based on Bertrand's teachings that antisense oligonucleotides are only able to provide a partial inhibition of gene

expression. Instead, Sherr only partially inhibits p18 gene expression, which leads to cell proliferation. As set forth in Bertrand, siRNA provides an almost complete inhibition of gene expression. Thus, the siRNA used in the claimed method completely or almost completely inhibits p18 expression, which promotes or stimulates self-renewal in a population of stem cells.

In addition, the Examiner asserts that based on the teachings of Bertrand "that one of ordinary skill in the art, at the time of the invention was made, would have found it prima facie obvious to use either siRNA or antisense oligonucleotides to block expression of the p18 gene in the method of Sherr."

Applicants submit that it has been held that "if a proposal for modifying the prior art in an effort to attain the claimed invention causes the art to become inoperable or destroys its intended function, then the requisite motivation to make the modification would not have existed." See *In re Fritch*, 972 F.2d at 1265, 23 USPQ.2d at 1783. Furthermore, MPEP §2143.01 (VI) states "if the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious." See *In re Ratti*, 270 F.2d 810, 123 USPQ 349 (CCPA 1959).

Applicants submit that it would be improper to combine the teachings of Sherr and Bertrand and use siRNA instead of antisense oligonucleotides in the Sherr reference because the siRNA would destroy the intended function and/or change the method of controlling proliferation in Sherr. As set forth herein, Sherr uses antisense oligonucleotides to inhibit p18 expression. However, antisense oligonucleotides inhibit gene expression far less compared to siRNA as set forth herein. By using siRNA the

inhibition of p18 gene expression would improve dramatically and possibly change the overall process of controlling proliferation to instead promoting self-renewal. Thus, Sherr's method of controlling proliferation would be changed or destroyed. Indeed, replacing antisense oligonucleotides with siRNA in the Sherr method of controlling proliferation would require wholesale modifications of function and operation in a manner that is neither contemplated nor intended by Sherr.

Furthermore, An provides no teaching that, when combined with Sherr and Bertrand that would lead one of ordinary skill in the art to the Applicants' claimed method of promoting or stimulating self-renewal. Indeed, the Examiner only cites An for its teachings of delivering siRNA molecules to cells using lentiviral vectors.

In addition, Walters provides no teaching that, when combined with Sherr, Bertrand, and An that would lead one of ordinary skill in the art to the Applicants' claimed method of promoting or stimulating self-renewal. Indeed, the Examiner only cites Walters for its teachings of delivering siRNA molecules to cells using electroporation.

For at least the reasons set forth herein, Applicants submit that the combined teachings of Sherr, Bertrand, An and Walters do not establish a prima facie case for obviousness because one of ordinary skill in the art reviewing the combined teachings of the references would not be inclined to arrive at the claimed method of promoting or stimulating self-renewal comprising:

1. a population of human stem cells;
2. siRNA;
3. reduction of p18 levels.

Accordingly, Applicants respectfully request withdrawal of the rejection of claims 1-3 and 23-25 under 35 U.S.C. §103(a) in view of Sherr further in view of Bertrand, An and Walters.

H) Rejection of Claims 1-3, 6-8 and 23-28 under 35 U.S.C. §103(a)

Previous claims 1-3, 6-8, and 23-28 were rejected under 35 U.S.C. §103(a) for assertedly being obvious in view of Sherr further in view of Bertrand, An, and Walters further in view of U.S. Patent No. 5,837,507 to Largman *et al.* (hereinafter, "Largman"). Applicants traverse this rejection for at least the reasons set forth herein.

Sherr in view of Bertrand, An, and Walters is clearly distinguished from claims 1-3, 6-8, and 23-28 for at least the reasons set forth in *Section G.* Furthermore, Largman provides no teaching that, when combined with Sherr, Bertrand, An, and Walters, that would lead one of ordinary skill in the art to the Applicants' claimed method of promoting or stimulating self-renewal. Indeed, the Examiner only cites Largman for its teachings of reintroducing hematopoietic stem cells into a human.

Thus, it is respectfully submitted that claims 1-3, 6-8, and 23-28 are not obvious in view of the combined teachings of the Sherr, Bertrand, An and Walters in view of Largman. Accordingly, Applicants respectfully request withdrawal of the rejection of claims 1-3, 6-8, and 23-28 under 35 U.S.C. § 103(a).

I.) New Claims 29-39

New claims 29-39 recite novel and non-obvious methods of promoting or stimulating self-renewal of a population of human stem cells by reducing intracellular

levels of p18. The cited references, Sherr, Bertrand, An, Walters, and Largman do not teach or suggest those recited methods of promoting or stimulating self renewal.

Thus, Applicants believe that claims 29-39 are patentable in view of the cited prior art. Accordingly, consideration and allowance of new claims 29-39 is respectfully requested.

CONCLUSION

Applicants respectfully submit that claims 1-3, 6-8 and 23-28 recite novel and non-obvious methods of promoting or stimulating self-renewal. Applicants believe that these claims define over the prior art of record and are in proper form for allowance. In view of the foregoing, Applicants respectfully submit that the Subject Application is in condition for allowance. Accordingly, reconsideration of the rejections and allowance of claims 1-2, 6-7, 23-24, 26-27 and 29-39 at an early date are earnestly solicited.

Applicants do not otherwise concede, however, the correctness of the rejections with respect to any of the dependent claims not discussed above. Accordingly, Applicants hereby reserve the right to make additional arguments as may be necessary to further distinguish the dependent claims from the cited references based on additional features contained in the dependent claims that were not discussed above. A detailed discussion of these differences is believed to be unnecessary at this time in view of the differences in the claims discussed herein.

Applicants further submit that canceled claims 3-5 and 8-22 may be filed in a subsequent continuation application.

If the undersigned can be of assistance to the Examiner in addressing any additional issues to advance the application to a condition of allowance, please contact the undersigned at the number set forth below.

Respectfully submitted,

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